



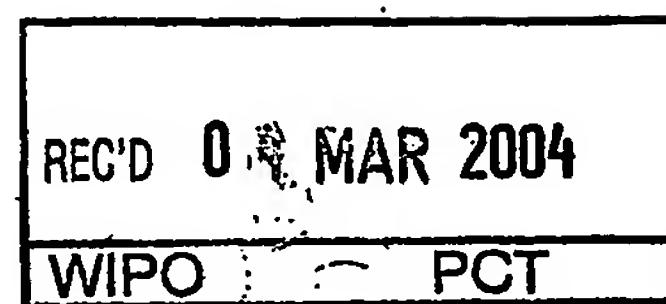
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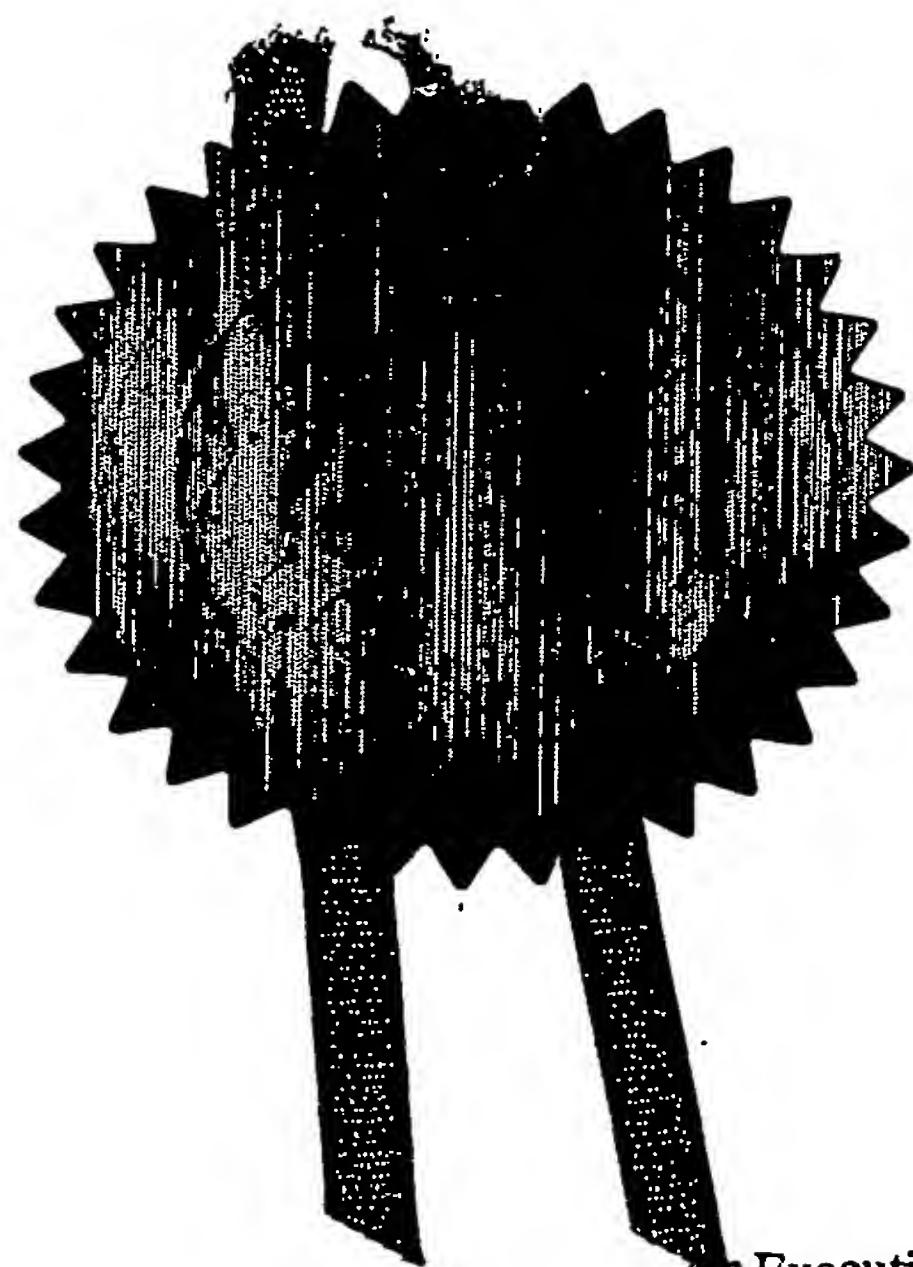
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CRF-GB-2-307

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3. Full name, address and postcode of the or of each applicant (underline all surnames)

D. Collen Research Foundation, a/o Dienst Prof. Collen, Campus Gasthuisberg, Herestraat 49, 3000 Leuven

Represented by Prof Collen, Chairman

Patent's ADP number (if you know it)

8508327002

If the applicant is a corporate body, give the country/state of its incorporation

Belgium

4. Title of the invention

Haemostatic and thrombotic

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

D. Collen Research Foundation

care of:

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London SW5 0HN

UK

8508335001

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Prof. D. Collen, Chairman

1/ We request the grant of a patent on the basis of this application.

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DUPLICATE

~~Haemostasis and thrombosis~~

FIELD OF THE INVENTION

5

This invention relates to the control of the primary haemostasis and the modulation of platelet number and platelet function in a subject. More specifically the invention relates to use of pituitary adenylyl cyclase activating peptide (PACAP), its derivatives, mimetics or inhibitors to modulate the primary haemostasis or thrombocytopoiesis or the use of 10 agonists or antagonists of the platelet receptor, VPCA1, to modulate the primary haemostasis or thrombocytopoiesis.

BACKGROUND OF THE INVENTION

15 The present invention relates to a new method for prevention and treatment of either thrombosis or bleeding based on administration of pituitary adenylyl cyclase activating peptide (PACAP) mimetics or inhibitors respectively. The initial response to interruption of continuity of a blood vessel is defined as primary haemostasis. Platelets play a major role in the pathophysiology of primary haemostasis.

20 The clinical importance of platelets became first obvious when thrombocytopenic patients who later on were diagnosed as having immune mediated thrombocytopenia (ITP) had purpura. Platelets participate in haemostasis by sealing vascular injuries and by fostering the process of blood coagulation. Not only the number of the platelets is important (thrombocytopenia for whatever reason) but also their intrinsic function upon 25 activation: platelet shape change, adhesion, aggregation and secretion are prerequisites for normal haemostasis. Congenital or acquired disorders interfering with one of its function can lead to mild to even severe bleeding problems.

30 Prevention and treatment of bleeding in patients with thrombocytopenia or thrombocytopathia is therefore based on platelet transfusion or medication interfering with platelet number and/or function.

Platelets play also a rôle in the development of arterial thrombosis. Disruption of the endothelial cell lining of the vessels exposes adhesive proteins within the subendothelial matrix, leading to platelet attachment. Thereafter platelet spreading occurs as well as platelet secretion. The secretion of the content of platelet granules can stimulate 5 circulating platelets to acquire new adhesive properties. Finally, stimulated platelets interact with one another during platelet aggregation and a platelet-rich thrombus is formed, which can compromise the patency of blood vessels. Furthermore activated platelets accelerate the rate at which coagulation proteins are activated: phospholipids on the platelet surface facilitate thrombin generation and fibrin strand formation.

10 Arterial and venous thrombosis and their complications including ischemic stroke, acute myocardial infarction and venous thromboembolism, represent the major cause of morbidity and mortality in the developed countries. Prevention and treatment of thrombosis are therefore based on administration of antiplatelet drugs, anticoagulants or thrombolytic therapy or combinations of them.

15 The pituitary Adenyllyl Cyclase Activating Peptide (PACAP 1-38) is a 38-amino acid peptide that was first isolated from ovine hypothalamic extracts on the basis of its ability to stimulate cAMP formation in anterior pituitary cells (1,2). PACAP is part of the vasoactive intestinal polypeptide (VIP)- glucagon- growth hormone releasing factor- 20 secretin superfamily. Its role in biology is probably crucial, since the sequence of PACAP is highly conserved during the evolution from protochordate to mammals. PACAP is widely expressed: in the central and peripheral nervous system, the urogenital system, the gastro-intestinal tract, several endocrine glands. Also PACAP receptors are widely distributed (2). Two classes of PACAP binding sites have been characterized on their 25 relative affinities for PACAP and VIP: type I binding sites with high affinity for PACAP ($K_d = 0.5 \text{nM}$) and much lower affinity for VIP ($K_d > 500 \text{nM}$) and type II binding sites, which are widely distributed in various peripheral organs, characterized with similar affinities for PACAP and VIP ($K_d = 1 \text{nM}$). Molecular cloning of PACAP receptors has demonstrated the existence of three distinct receptor subtypes that are abundantly spread 30 in many tissues: the PACAP-specific PAC1 receptor, coupled to different signal transduction systems, and two PACAP: VIP-indifferent VPAC1 and VPAC2 receptors,

which are primarily coupled to adenylyl cyclase. We found that human platelets do express the VPAC1 receptor. The exact biological and pharmacological function of PACAP has presently been investigated in many organs and tissues as in endocrine glands, central nervous system, respiratory system, cardiovascular system and 5 gastrointestinal tract. Although also extensive studies have been performed on its function in the immune system, no data are available concerning its function on haemostasis.

We have recently found that PACAP has an important function in primary haemostasis; platelet number as well as platelet function are highly influenced by PACAP.

10

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Some terms and definitions in the present invention will be defined first.

15 The term "pharmaceutically acceptable" is used adjectively herein to mean that the modified noun is appropriate for use in a pharmaceutical product. The term "treatment" refers to any process, action, application, therapy, or the like, wherein a mammal, including a human being, is subject to medical aid with the object of improving the mammal's condition, directly or indirectly.

20

As used herein, "thrombocytopenia" is a disorder in which the platelet levels in the affected individual fall below a normal range of platelet numbers for that individual. Thrombocytopenia includes infection-induced thrombocytopenia, treatment-induced thrombocytopenia, others. Infection-induced thrombocytopenia is a disorder 25 characterized by a low level of platelets in peripheral blood, which is caused by an infectious agent such as a bacteria or virus. Treatment induced thrombocytopenia is a disorder characterized by a low level of platelets in peripheral blood which is caused by therapeutic treatments such as gamma irradiation, therapeutic exposure to radiation, cytotoxic drugs, chemicals containing benzene or anthracene and even some commonly 30 used drugs such as chloramphenicol, thiouracil, and barbiturate hypnotics. Other types of thrombocytopenia comprise disorders characterized by a low level of platelets in

peripheral blood, which is caused by any mechanism other than infectious agents or therapeutic treatments causing thrombocytopenia. Factors causing this type of thrombocytopenia include, but are not limited to, rare bone marrow disorders such as congenital amegakaryocytic hypoplasia and thrombocytopenia with absent radii (TAR syndrome), an increase in spleen size, or splenomegaly, caused by portal hypertension, secondary to liver disease, or macrophage storage disorders such as Gauchers disease, autoimmune disorders such as idiopathic or immune thrombocytopenic purpura (ITP), vasculitis, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura (TTP), disseminated intravascular coagulation (DIC) and prosthetic cardiac valves. ITP is by far 5 the most frequent type in this group of thrombocytopenias. A subject having thrombocytopenia is a subject having any type of thrombocytopenia. In some embodiments the subject having thrombocytopenia is a subject having non-chemotherapeutic induced thrombocytopenia. A subject having non-chemotherapeutic thrombocytopenia is a subject having any type of thrombocytopenia but who is not 10 undergoing chemotherapy. In other embodiments the subject is a subject having chemotherapeutic induced thrombocytopenia, which includes any subject having 15 thrombocytopenia and being treated with chemotherapeutic agents.

As used herein, "a subject at risk of developing thrombocytopenia" is a subject who has a 20 high probability of acquiring or developing thrombocytopenia. For example, a patient with a malignant tumour who is prescribed a chemotherapeutic treatment is at risk of developing treatment induced thrombocytopenia and a subject who has an increased risk of exposure to infectious agents is at risk of developing infection-induced thrombocytopenia.

25

"Increased levels of active platelets" means that platelet number is higher than compared with the mammal as compared with the mammal not treated with an PACAP inhibitor of the invention. An amount effective to increase platelet counts in the subject is an amount, which causes an increase in the amount of circulating platelet levels. The actual levels of 30 platelets achieved will vary depending on many variables such as the initial status of the immune system in the subject, i.e., whether the subject has mild to severe

thrombocytopenia (e.g., resulting from an autoimmune disease or splenic sequestration). In general, the platelet levels of a subject who has severe thrombocytopenia will initially be very low. Any increase in the platelet levels of such a subject, even increases to a level that is still below a normal level, can be advantageous to the subject.

5

The term "a compound that inhibits the expression" refers here to gene expression and thus to the inhibition of gene transcription and/or translation of a gene transcript (mRNA) such as for example the PACAP gene. Preferably said inhibition is at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or even higher.

10

The term "a compound that inhibits the activity" refers to the protein that is produced such as PACAP or its receptor, in this invention preferably the VPAC1 receptor. The inhibition of activity leads to a diminished interaction (e.g. in the case of PACAP or VIP with the VPAC (1) receptor) with its receptor and an inhibition of signal transduction).

15 Preferably said inhibition is at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or even higher.

20 ANTI-THROMBOTIC AGENTS

An important embodiment of present invention relates to compositions comprising an activator of PACAP and/or a PACAP receptor agonist, preferably a VPAC(1) receptor agonist for treatment (particularly the prevention or suppression) of platelet 25 hyperfunction in a subject.

It can be a pharmaceutical composition, which comprises an amount of PACAP or a mimetic thereof effective for blocking or preventing thrombosis in a subject, and a pharmaceutically effective carrier. This pharmaceutical composition can be used to treat a subject having a thrombus or at risk of thrombus formation or to manufacture a medicament to treat a subject having a thrombus or at risk of thrombus formation.

PACAP receptor selective agonists are, for instance, the compounds of the group consisting of the PACAP, VIP, [K(15),R(16),L(27)]VIP(1-7)/GRF(8-27), [R(16)]ChSn), (Lundberg et al., Endocrinology 2001 Jan. 142(1): 339 – 47; Lema Kisoka R et al. Peptides 2001 Dec; 22 (12): 2155-62), [(11,22,28)Ala]-VIP (Anderson C.M. et al. Br. J. Pharmacol. 2003 Feb., 138 (4): 564 – 73).

On the other hand, the 38-amino acid form of PACAP (PACAP38), HSDGIFTDSYSRYRKQMAVKKYLAALGKRYKQRVKNK-NH₂ and PACAP27, a 27-residue alpha-amidated polypeptide, HSDGIFTDSYSRYRKQMAVKKYLAAL-NH₂ are potent agonists of PACAP/VIP receptors. Based on their binding affinity and adenylyl cyclase stimulating properties, PACAP 2-38, PACAP 2-27, AcHIS1 PACAP1-27, Ala2 PACAP(1-27) have also been demonstrated to be potent agonists of PACAP/VIP receptors (Vaudry D. et al, Pharmacological reviews Vol. 52, No. 2: 269-324).

Amino acid substitutions and additions of a fatty acyl moiety have led to the development of lipophilic VIP derivatives that exhibit enhanced potency and specificity for VPAC receptors (Gozes and Fridkin, J. Clin. Invest 90: 810 – 814, 1992; Gozes et al, Cell Mol. Neurobiol 15: 675-687, 1995; Gourlet et al. Eur. J. Pharmacol 354: 105-111, 1998).

Agonists of the type II PACAP receptor, VPAC(1) are for instance compounds of the group consisting of [R(16)]-PACAP(1-23) (Van Rampelbergh et al. Br. J. Pharmacol. 2000 Jun; 130 (4): 819 – 26), [(L22)]-VIP (Van Rampelbergh et al. Br. J. Pharmacol. 2000 Jun; 130 (4): 819 – 26), (Lys15, Arg16, Leu27)VIP1-7 GRF8-27 (Moody TW et al Ann-N.Y. Acad. Sci. 2000; 921: 26-32).

Present invention has now demonstrated that a pharmaceutical composition, which comprises an effect amount of above mentioned PACAP/VIP receptor agonist and a

pharmaceutically effective carrier can be used decrease platelet hyperfunction and/or for blocking or preventing thrombosis formation in a subject. Such pharmaceutical composition can be to manufacture a medicament to treat a subject having a thrombus or at risk of thrombus formation.

5

The invention provides thus compositions and methods useful for inhibiting, suppressing or ameliorating platelet hyperfunction in mammals, including humans. The invention applies to human and veterinary applications. The inventive composition and method have been shown to be especially effective in preventing thrombosis formation. A new 10 class of pharmaceutical compositions and methods of treatment and prevention of thrombosis and thrombosis related injury and disease is provided.

15 The subject of present invention also provides a pharmaceutical composition, which comprises an activator of PACAP or PACAP receptor agonist, which in combination with an other antithrombotic agent, in an amount and proportion for enhancing the action of the other antithrombotic agent (e.g. aspirin, or an agent which blocks glycoprotein IIbIIIa, heparin, warfarin, coumarin derivatives, thrombin inhibitors, or Factor Xa inhibitors) to prevent clotting or dissolve clots which have already formed.

20 The subject of present invention also provides a pharmaceutical composition, which comprises an activator of PACAP or PACAP receptor agonist, which in combination with an other antithrombotic agent, in an amount and proportion for enhancing the action of thrombolytic (e.g. staphylokinase, streptokinase, urokinase, tissue plasminogen activator, plasmin, mini- or microplasmin) to dissolve clots which have already formed

25

30 In addition, the present invention finds utility in other contexts where prevention of platelet hyperactivity is a significant concern, e.g. acute coronary syndrome, myocardial infarction, acute myocardial infarction, unstable angina, refractory angina, peripheral vascular disease, occlusive coronary thrombus occurring post-thrombolytic therapy or post-coronary angioplasty, a thrombotically mediated cerebrovascular, syndrome, embolic stroke, thrombotic stroke, transient ischemic attacks, venous thrombosis, deep

venous thrombosis, pulmonary embolus, coagulopathy, disseminated intravascular coagulation, thrombosis following angioplasty, restenosis following angioplasty, thrombosis following carotid endarterectomy, thrombotic thrombocytopenic purpura, thromboangiitis obliterans, thrombotic disease associated with heparin-induced 5 thrombocytopenia, thrombotic complications associated with extracorporeal circulation, thrombotic complications associated with instrumentation such as cardiac or other intravascular catheterization, intra-aortic balloon pump, coronary stent, atherosclerosis, or cardiac valve, disorder is characterized by transient ischemic attacks, and conditions required the fitting of prosthetic devices.

10

Regarding the method for blocking or preventing thrombosis in a subject, this invention provides that the subject may be a human. The human may be a patient. The subject may also include other mammals; examples include dogs, cats, horses, rodents, or pigs, rabbits, among others.

15

ANTI-HAEMORRHAGIC AGENTS

20 This invention also relates to compositions for treatment (particularly the prevention or suppression) of platelet hypofunction in a subject, more specifically a composition comprising a molecule selected from the list consisting of an antisense molecule, a small molecule, an antibody, a ribozyme, a transdominant receptor, a tetrameric peptide in an effective amount to inhibit the expression and/or activity of a PACAP gene (PACAP 25 inhibitor), the activity of the VRAC(1) receptor, or in an effective amount to inhibit the expression and/or activity of a VIP gene (VIP inhibitor). The compositions for inducing platelet production, comprising an effective quantity of a PACAP inhibitor or VIP inhibitor can be in admixture with pharmaceutically acceptable diluents, carriers or excipients.

30

This property of stimulating platelet production of the molecule should render it a useful adjunct in therapy of patients suffering from acute thrombocytopenia, for example, as a

result of chemo- or radiotherapy of various cancers. Currently, such patients are at grave risk when circulating platelet levels are depressed to levels wherein thrombogenesis is precluded.

- 5 The invention provides compositions and methods useful for activating thrombocytopoiesis in mammals, including humans. The invention applies to human and veterinary applications. The inventive composition and method have been shown to be especially effective in treating platelet hypofunction.
- 10 In addition, the present invention finds utility in other contexts, e.g., Radiation or chemotherapeutic treatment, where prevention or treatment of thrombocytopenia, anemia and neutropenia is a significant concern. The depletion of hematopoietic precursors in the bone marrow associated with chemotherapy and irradiation result in hemorrhagic and infectious complications. Severe suppression of the hematopoietic system is a major factor in limiting chemotherapy use and dose escalation.
- 15

The invention in one aspect is a method for increasing platelet counts in a subject having thrombocytopenia or subject at risk of developing thrombocytopenia by administering to the subject a PACAP or a VIP inhibitor.

- 20
- In one embodiment the invention provides the use of a compound that inhibits the expression and/or activity of PACAP gene for the manufacture of a medicament for treatment of platelet hypofunction or low platelet levels. This is based on the finding that platelet hypofunction can be treated (activated) or the platelet level can be increased by 25 inhibiting the expression and/or activity of PACAP.

Another embodiment of the invention provides the use of a compound that inhibits the expression and/or activity of VIP gene for the manufacture of a medicament for treatment platelet hypofunction or low platelet levels.

30

Compounds useful for exercising the thrombocytopoietic treatment or the manufacturing of a medicament for platelet hypofunction of present invention are thus compounds that inhibit the activity of PACAP or VIP and more specifically that antagonise PACAP platelet receptor. For instance several compounds, e.g. (poly)peptides are known to be 5 PACAP receptor antagonists as for instance the compounds of the group consisting of max.d.4.5 (Sakashita Y et al., Br. J. Pharmacol 2001 Apr., 132 (8): 1769 – 76), PACAP6-38 (Tohei et al, Neuroendocrinology 2001 Jan., 73(1): 68-74). Compounds that are known to have type II PACAP receptor antagonists properties are several N-terminal truncated or substituted VIP peptide such as [4Cl-D-Phe6, Leu17]VIP, VIP(10-28) 10 (Pandol et al., AM. J. Physiol. 250: G553 – G557 1986; Turner et al., Peptides 7: 849-854, 1986; Gozes et al., 1995; Gourlet et al., 1997a), cyclic lactam analogs of PACAP (Gitar et al., Peptides 15: 461 – 466, 1994). VPAC(1) receptor selective antagonists known are for instance [AchHis(1), D-Phe(2), Lys(15), Leu(17)]VIP(3-7)/GRF(8-27) 15 (Lema Kisoka R et al. Peptides 2001 Dec, 22 (12): 2155 - 62), neutralising antibodies against VPAC(1) or aptamers (3-dimensional nucleic acids that bind to molecular targets in a manner similar to antibodies) that bind to VPAC(1) receptor or against PACAP thereby neutralising its activity.

The invention is directed to the usage of molecules that act as inhibitors (or antagonists) 20 of PACAP or its receptor VPAC(1) such as antibodies and functional fragments derived thereof, anti-sense RNA and DNA molecules (e.g. polynucleotide sequences), ribozymes that function to inhibit the translation of PACAP.

Small molecules can also interfere by binding on the promoter region of PACAP and 25 inhibit binding of a transcription factor on said PACAP promoter region so that no PACAP mRNA is produced.

An embodiment of the present invention relates to the usage of molecules which comprise a region that can specifically bind to PACAP or to its receptor (VPAC(1) 30 receptor) and consequently said molecules interfere with the binding of PACAP and/or VIP to its VPAC(1) receptor interfering with the signal transduction of PACAP and/or

VIP and said molecules can be used for the manufacture of a medicament for treatment of platelet hypofunction. Thus more specifically the invention also relates to molecules that neutralize the activity of PACAP and/or VIP by interfering with its synthesis, translation, dimerisation, receptor-binding and/or receptor-binding-mediated signal transduction. By 5 molecules it is meant peptides, tetrameric peptides, proteins, organic molecules, mutants of the PACAP or VIP, soluble receptors of VPAC(1) and any fragment or homologue thereof having the same neutralizing effect as stated above.

Also, the molecules in this invention comprise antagonists of PACAP such as anti-10 PACAP antibodies and functional fragments derived thereof, anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of PACAP, all capable of interfering/or inhibiting the VPAC(1) signal transduction. By synthesis it is meant transcription of PACAP. Small molecules can bind on the promoter region of PACAP and inhibit binding of a transcription factor or said molecules can bind said transcription 15 factor and inhibit binding to the PACAP-promoter. By PACAP it is meant also its isoforms, which occur as a result of processing of PACAP precursors. As a result of precursor processing PACAP two isoforms are known, PACAP-38 and PACAP-27.

In another the invention provides the use of molecules that inhibit the expression and/or 20 activity of VIP for the manufacture of a medicament for treatment of platelet hypofunction. Thus more specifically the invention relates to the use of molecules that neutralize the activity of VIP by interfering with its synthesis, translation, receptor-binding and/or receptor-binding-mediated signal transduction. By molecules it is meant peptides, tetrameric peptides, proteins, organic molecules, having the same neutralizing 25 effect as stated above. Also, in this invention the molecules comprise antagonists of VIP such as anti-VIP antibodies and functional fragments derived thereof, anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of VIP, all capable of interfering/or inhibiting the VIP signal transduction. By synthesis it is meant transcription of VIP. Small molecules can bind on the promoter region of VIP and inhibit 30 binding of a transcription factor or said molecules can bind said transcription factor and inhibit binding to the VIP-promoter so that there is no expression of VIP.

The term 'antibody' or 'antibodies' relates to an antibody characterized as being specifically directed against VPAC(1), PACAP, VIP or any functional derivative thereof, with said antibodies being preferably monoclonal antibodies; or an antigen-binding 5 fragment thereof, of the F(ab')2, F(ab) or single chain Fv type, or any type of recombinant antibody derived thereof. These antibodies of the invention, including specific polyclonal antisera prepared against VPAC(1), PACAP, VIP or any functional derivative thereof, have no cross-reactivity to others proteins. The monoclonal antibodies of the invention can for instance be produced by any hybridoma liable to be formed 10 according to classical methods from splenic cells of an animal, particularly of a mouse or rat immunized against VPAC(1), PACAP, VIP or any functional derivative thereof, and of cells of a myeloma cell line, and to be selected by the ability of the hybridoma to produce the monoclonal antibodies recognizing VPAC(1), PACAP, VIP or any functional derivative thereof which have been initially used for the immunization of the animals. 15 The monoclonal antibodies according to this embodiment of the invention may be humanized versions of the mouse monoclonal antibodies made by means of recombinant DNA technology, departing from the mouse and/or human genomic DNA sequences coding for H and L chains or from cDNA clones coding for H and L chains. Alternatively the monoclonal antibodies according to this embodiment of the invention 20 may be human monoclonal antibodies. Such human monoclonal antibodies are prepared, for instance, by means of human peripheral blood lymphocytes (PBL) repopulation of severe combined immune deficiency (SCID) mice as described in PCT/EP 99/03605 or by using transgenic non-human animals capable of producing human antibodies as described in US patent 5,545,806. Also fragments derived from these monoclonal 25 antibodies such as Fab, F(ab')2 and ssFv ("single chain variable fragment"), providing they have retained the original binding properties, form part of the present invention. Such fragments are commonly generated by, for instance, enzymatic digestion of the antibodies with papain, pepsin, or other proteases. It is well known to the person skilled in the art that monoclonal antibodies, or fragments thereof, can be modified for various 30 uses. An appropriate label of the enzymatic, fluorescent, or radioactive type can label the antibodies involved in the invention.

Small molecules, e.g. small organic molecules, and other drug candidates can be obtained, for example, from combinatorial and natural product libraries.

5 Random peptide libraries, such as the use of tetrameric peptide libraries such as described in WO0185796, consisting of all possible combinations of amino acids attached to a solid phase support may be used in the present invention. Also transdominant-negative mutant forms of PACAP-receptors (e.g. a transdominant-negative receptor of VPAC(1) can be used to inhibit the signal transduction of PACAP or VIP. Also within the scope of the
10 invention is the use of oligoribonucleotide sequences that include anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of VPAC(1) mRNA or PACAP mRNA or VIP mRNA. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from
15 the translation initiation site. Ribozymes are enzymatic RNA molecules capable of catalysing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridisation of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently
20 catalyse endonucleolytic cleavage of VPAC(1) RNA, PACAP RNA or VIP RNA sequences. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene
25 containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as for
30 example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding the

antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors, which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize anti-sense RNA constitutively or inducible, depending on the promoter used, can be 5 introduced stably into cell lines.

The term 'medicament to treat' relates to a composition comprising molecules as described above and a pharmaceutically acceptable carrier or excipient (both terms can be used interchangeably) to treat diseases as indicated above. Suitable carriers or excipients 10 known to the skilled man are saline, Ringer's solution, dextrose solution, Hank's solution, fixed oils, ethyl oleate, 5% dextrose in saline, substances that enhance isotonicity and chemical stability, buffers and preservatives. Other suitable carriers include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition such as proteins, polysaccharides, polylactic acids, 15 polyglycolic acids, polymeric amino acids and amino acid copolymers. The 'medicament' may be administered by any suitable method within the knowledge of the skilled man. The preferred route of administration is parenterally. In parental administration, the medicament of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with the 20 pharmaceutically acceptable excipients as defined above. However, the dosage and mode of administration will depend on the individual. Generally, the medicament is administered so that the protein, polypeptide, peptide of the present invention is given at a dose between 1 μ g/kg and 10 mg/kg, more preferably between 10 μ g/kg and 5 mg/kg, most preferably between 0.1 and 2 mg/kg. Preferably, it is given as a bolus dose. 25 Continuous infusion may also be used and includes continuous subcutaneous delivery via an osmotic minipump. If so, the medicament may be infused at a dose between 5 and 20 μ g/kg/minute, more preferably between 7 and 15 μ g/kg/minute.

Another aspect of administration for treatment is the use of gene therapy to deliver the 30 above mentioned anti-sense gene or functional parts of the VPAC(1) gene, PACAP gene or VIP gene or a ribozyme directed against the VPAC(1) mRNA, PACAP mRNA, VIP

mRNA or a functional part thereof or a genetic construct encoding a transdominant-negative mutant form of VPAC(1)-receptors. Gene therapy means the treatment by the delivery of therapeutic nucleic acids to patient's cells. This is extensively reviewed in Lever and Goodfellow 1995; Br. Med Bull., 51, 1-242; Culver 1995; Ledley, F.D. 1995.

5 Hum. Gene Ther. 6, 1129. To achieve gene therapy there must be a method of delivering genes to the patient's cells and additional methods to ensure the effective production of any therapeutic genes. There are two general approaches to achieve gene delivery; these are non-viral delivery and virus-mediated gene delivery.

10

Brief description of the drawings

Figure 1. *Family presentation.* Squares, male; circles, female; filled symbols, affected individuals; open symbols, unaffected individuals. The proband is indicated with an arrow. Black filled symbols represent members with severe mental retardation and a partial trisomy 18p and monosomy 20p, striped boxes represent members with borderline IQ and the balanced translocation t(18;20) (p21,p13) while question marks stand for members with unexplained mental retardation but unknown karyotype.

20 Figure 2. *Platelet aggregation and adenylyl cyclase activity.* A. $IC_{50} \pm$ accuracy values for indicated individuals and the mean IC_{50} value for Iloprost in the platelet aggregation inhibition test with 2 μ g/ml collagen in 22 controls were calculated. A significantly ($P \leq 0.03^*$ or $P \leq 0.0076^{**}$) lower IC_{50} value indicates a Gs hyperfunction. The right column of this table illustrates the significantly decreased response to collagen (μ g/ml) for 25 respectively IV:5 and V:3 ($P \leq 0.003^*$) versus V:4 and VI:1 ($P \leq 0.0001^{**}$) compared to 10 controls or IV:6. EC_{50} is expressed as collagen concentration that induces aggregation with amplitude 50 % of maximal aggregation. B. Measurements of cAMP levels under basal conditions performed in duplicate (left panel) or after stimulation with Iloprost (1 ng/ml) for various time intervals (right panel) in platelets from VI:1 (■) or an unrelated control (*). C. Measurements of cAMP levels under basal conditions performed in 30 duplicate (left panel) or after stimulation with isoproterenol (1 μ M) for various time

intervals (right panel) in fibroblasts from VI:1 (■), a patient with trisomy 18 (▲) or an unrelated control (*). All cAMP measurements were performed in the presence of the phosphodiesterase inhibitor IBMX (400 μ M).

5 **Figure 3.** Localization of the PACAP gene by FISH. Two color FISH with probe Y841C3 (green) and two centromeric probes for chromosome 18 and 20 (red) for patient VI:1 and his mother V:3. The arrows point to the PACAP signals.

10 **Figure 4.** PACAP detection in fibroblasts and plasma. A. Semi-quantitative RT-PCR using 20 cycles showed PACAP(1-38) overexpression in fibroblasts from patient VI:1 compared with two controls. β -actin is the internal control. B. PACAP detections by ELISA in plasma from citrate (left panel) or ACD (right panel) blood show pronounced or moderately increased PACAP level in respectively VI:1 (■) and V:4 (▲) or IV:5 (●) and V:3 (◆) versus a citrated plasma pool (*) or IV:6 (◇). C. Collagen induced aggregation of control platelets in plasma from a control or from patient IV:1 (two experiments shown).

15 **Figure 5.** Role of PACAP(6-38) in platelet aggregation. A. Dose-dependent stimulatory effect of PACAP(6-38) on collagen-induced (0.2 μ g/ml) platelet aggregation. B. The platelet aggregation inhibition test with collagen (2 μ g/ml) and different concentrations of Iloprost (ng/ml) as indicated in the absence (left panels) and presence (right panels) of PACAP(6-38) for a control (upper panels) or patient VI:1 (lower panels).

20 **Figure 6.** Effect of anti-PACAP antibodies in mice. Platelet aggregation was performed in PRP pooled from five mice of each group with 250×10^3 plt/ μ l. A. Stimulatory effect of a polyclonal anti-PACAP antibody (10 μ g/ml) on collagen-induced (0.35 μ g/ml) platelet aggregation. B. The platelet aggregation inhibition test with collagen (2 μ g/ml) and preincubation of Iloprost (10 ng/ml) for mice injected with the indicated antibodies. C. Platelet aggregation induced with a low concentration of collagen (0.2 μ g/ml) for mice 25 injected with the indicated antibodies.

Figure 7. The mean platelet number per μ l for mice (n=5) injected with either polyclonal anti-PACAP (A) or an irrelevant anti- β 2-glycoprotein I (B) antibody was determined 14 days after the first antibody injection.

5 Figure 8. Mean platelet number +/- SD for mice (n=5) injected with either polyclonal anti-PACAP (A) or anti-vWF (75H4B12) (B) antibody determined at the indicated days.

Figure 9. Mean platelet number/ μ l +/- SD for mice (n=5) injected with either polyclonal anti-PACAP (A) or an irrelevant anti-XL α s (B) polyclonal antibody determined at the 10 indicated days. Data represent separated experiments A and B.

15 The following examples more fully illustrate preferred features of the invention, but are not intended to limit the invention in any way. All of the starting materials and reagents disclosed below are known to those skilled in the art, and are available commercially or can be prepared using well-known techniques.

PACAP overexpression in patients

Patient descriptions.

20 We describe a family characterised by an unbalanced segregation of the reciprocal translocation t(18;20) (p21;p13), of which different members suffer from unexplained mental retardation (Figure 1). The propositus (VI:1) is a 23-year-old boy with a hypogonadotropic hypogonadism and is followed for epilepsy, severe mental retardation, hyperactive behaviour and hypotonia. He has an increased bleeding tendency and the Ivy 25 bleeding time was markedly prolonged (> 15 minutes) but coagulation studies are normal. Electron microscopy of his platelets is completely normal but he presented on different occasions with a moderate thrombocytopenia, as his platelet count is always about $70-90 \times 10^3$ platelets/ μ l. His karyotype shows a partial trisomy 18p and monosomy 20p. His brother (VI:2), father (V:2) and maternal grandmother (IV:6) are phenotypically 30 normal, have no bleeding problems and have a normal karyotype. In contrast, his mother (V:3) and maternal grandfather (IV:5) have no obvious neurologic abnormalities but a

borderline IQ. They carry the balanced translocation t(18,20) (p21,p13). They don't have any obvious bleeding problems and have a normal platelet count. His 47-year-old uncle (V:4) also suffers from severe mental retardation, pronounced recurrent epistaxis and cryptorchidism. Furthermore, he frequently has gastric bleedings and his platelet count is 5 around 150×10^3 platelets/ μ l. He also has a partial trisomy 18p and monosomy 20p. Two other family members (IV:2 and V:1) are known with unexplained mental retardation but from these individuals no DNA samples or further clinical information are available.

Adenylyl cyclase activity in platelets and fibroblasts

10 The propositus VI:1 has disturbed platelet function with a gain-of-Gs activity measured by the platelet aggregation inhibition test, similar to what we described for patients with the XLas insertion (3,4). Platelets from the patients (VI:1 and V:4) with the partial trisomy 18p/monosomy 20p had a significantly increased sensitivity towards a Gs agonist, the prostacyclin analogue Iloprost (Figure 2A), while platelets from the family members (IV:5 and V:3) with the balanced translocation showed a moderately increased sensitivity. The IC_{50} value for member IV:6 with the normal karyotype is within the range of the IC_{50} values from 22 unrelated controls.

15 An important difference between patients from this family and the patients with the XLas insertion is their decreased sensitivity towards the platelet agonist collagen. 20 The collagen concentration to obtain 50 % aggregation for platelets from V:4 and VI:1 is significantly higher than for platelets from unrelated controls or the normal member IV:6 (Figure 2A). The reactivity of platelets from IV:5 and V:3 towards collagen is again mildly affected.

25 For patients with the XLas insertion, we demonstrated that the functional responses mediated by stimulation of Gs agonists are due to hyperactivity of adenylyl cyclase only when Gs-coupled receptors are stimulated (3,4). These patients had normal basal cAMP levels. We hypothesized that in the platelets of the propositus VI:1, adenylyl cyclase is already activated under basal conditions. In addition to an increased cAMP response to Iloprost, patient VI:1 indeed shows higher basal cAMP levels (Figure 2B). 30 We also performed cAMP measurements in fibroblasts from VI:1 and found a similar increased basal and stimulated cAMP response (Figure 2C).

PACAP(1-38) mRNA and protein overexpression

Patient VI:1 has a normal $G\alpha$ mRNA and protein expression level and the coding sequence for the $G\alpha$ gene and XL-exon1 were completely normal. Since this patient had

5 a partial trisomy 18p and monosomy 20p, these chromosomes were screened for candidate genes. Interestingly, measurement of the adenylyl cyclase activity in fibroblasts from an unrelated patient with a complete trisomy 18 showed similarly increased basal and stimulated cAMP levels (Figure 2C). The gene for PACAP (*ADCYAPI*) is located on chromosome 18p31-32 (5) and is a possible candidate since its active peptide, PACAP(1-38), stimulates $G\alpha$ -coupled receptors and thereby activates adenylyl cyclase. FISH analysis with YAC clone Y841C3 (6), that contains *ADCYAPI*, showed that the translocation results in three copies of the gene in patients VI:1 and V:4 (Figure 3).

Human skin fibroblasts express PACAP(1-38) and the PACAP type1-receptor (VPAC1) (7). PACAP(1-38) mRNA was overexpressed in fibroblasts from patient VI:1 (Figure 4A) by semi-quantitative RT-PCR. No PACAP mRNA was found in platelets by RT-PCR, probably due to their unstable RNA. However, we could show by western blot analysis that platelets express the VPAC1 receptor (data not shown). The active peptide PACAP (1-38) is mainly expressed in testis and brain but this peptide can cross the 15 blood-brain barrier and is stably transported in plasma through coupling with ceruloplasmin (8,9). PACAP(1-38) was detected in human plasma by ELISA and significantly higher levels were found in patients VI:1 and V:4, and moderately increased 20 levels in IV:5 and V:3, in contrast to a plasma pool of unrelated controls or IV:6 (Figure 4B). Platelet aggregation using washed control platelets resuspended in citrated plasma 25 from a control or patient VI:1, indicated that plasma from VI:1 inhibits the collagen induced aggregation (Figure 4C). This could be due to the increased amount of PACAP(1-38) in this plasma.

Role for PACAP in platelet aggregation

30 To determine the effects of the $G\alpha$ agonist PACAP(1-38) as an inhibitor of collagen induced platelet aggregation, we performed additional aggregations with its

antagonist PACAP(6-38). This recombinant peptide has a 10-100 times higher affinity for the VPAC1 receptor than PACAP(1-38) but seems not to activate adenylyl cyclase (2). PACAP(6-38) activates the collagen-induced platelet aggregation in a dose-dependent manner (Figure 5A). In the presence of PACAP(6-38), basal cAMP levels are 10-20 % lower (data not shown). The functional platelet aggregation inhibition test for a control person in the presence of PACAP (6-38) results in a Gs loss-of-function (Figure 5B). The influence of PACAP(6-38) on the platelet aggregation test for patient VI:1 was not that pronounced, probably because his PACAP plasma levels were too high.

10 Role for PACAP in platelet aggregation by studies in mice.

Functional platelet studies from patient VI:1 show that increased levels of PACAP(1-38) in plasma result in increased basal cAMP levels and a platelet hypofunction. The role of PACAP(1-38) in platelet function was also studied in mice by subcutaneous injection of polyclonal or monoclonal anti-PACAP antibodies. These 15 antibodies had a similar effect on platelet aggregation as the PACAP antagonist PACAP(6-38). Platelets incubated with anti-PACAP antibodies (10 µg/ml) show an enhanced response towards collagen stimulation (Figure 6A). Moreover, when mice were functionally tested by platelet aggregation 7 days after their last injection, mice treated with anti-PACAP antibodies show the opposite phenotype to that observed in patient 20 VI:1. In contrast to the treatment with the aspecific antibody against β2-glycoprotein I, anti-PACAP-treated mice show a weaker response towards activation of the Gs pathway and have an enhanced response towards collagen stimulation (Figure 6B,C).

25 Role of PACAP in thrombocytopenia

As the above described patient was also thrombocytopenic, we hypothesized that PACAP(1-38) or increased cAMP levels could lead to a defective megakaryocyte maturation. The role of PACAP(1-38) in thrombocytopoiesis was further studied in mice by subcutaneous injection of polyclonal or monoclonal anti-PACAP antibodies. The mice 30 injected with the anti-PACAP antibodies (group A, n=5) have furthermore increased

platelet numbers in contrast to the control group (group B, n=5) ($1194 \pm 237 \times 10^3$ plt/ μ l) versus $722 \pm 178 \times 10^3$ plt/ μ l, p= 0.01 - unpaired T-test) (Figure 7).

This experiment was repeated but now the platelet number was determined during the 5 experiment at different time points (days 0, 3, 7, 9, and 14) by tail bleeding (figure 8). Mice injected with anti-PACAP antibodies (at day 0, 3, and 7) and already show increased platelet numbers 3 days after antibody injection.

We studied the increased thrombocytopoiesis after pre-treatment with a polyclonal anti-10 PACAP antibody under conditions of chemically suppressed bone marrow by the agent busulfan. This was done by subcutaneous injection of mice with either a polyclonal anti-PACAP or a control polyclonal antibody (at days 0, 3, and 7) and afterwards an intraperitoneal injection of Busulfan (20 mg/kg) (at days 8 and 11). The platelet number was counted at different time points and we found that mice pretreated with the 15 polyclonal anti-PACAP antibody recovered more rapidly from their thrombocytopenic condition than the mice injected with the control antibody (Figure 9A,B).

Preparation of Monoclonal Antibodies against PACAP and its F(ab')2 or monovalent Fab fragments are for instance obtainable by:

20 *Immunosensitization and Cell Fusion:* A recombinant human PACAP(1-38) fusion protein, consisting of the amino acids encoded by the PACAP peptide 1 - 38 coupled to Glutathione S-transferase (GST) was expressed in *Escherichia coli* and purified by affinity chromatography on immobilized glutathione (Amersham Biosciences). Recombinant human PACAP(1-38) is mixed with an equal amount of an adjuvant, and an 25 obtained mixture is than subcutaneously administrated to Balb/c male mice (8 weeks old upon the start of immunization) in an amount corresponding to an amount of PACAP(1-38) of 100 μ g per 1 mouse (priming immunization). After about 21 days, immunization can be performed by subcutaneous administration in the same manner as described above (booster immunization). After 19 days or 30 days from the booster, the mice can 30 be administrated through their tail veins with 200 μ l of a preparation obtained by diluting human PACAP(1-38) with PBS (phosphate-buffered physiological saline) to have a

concentration of 250 µg/ml (final immunization). Spleens have than to be excised from the mice after about 3 days from the final immunization, and they have to be separated into single cells. Subsequently, the spleen cells should be washed with a proper medium, e.g. DMEM medium. On the other hand, suitable mouse myeloma cells (e.g. Sp2/0-Ag14) have to be collected in the logarithmic growth phase, and to be washed with a proper medium, e.g. DMEM medium. The spleen cells and the mouse myeloma cells have to be sufficiently mixed in a plastic tube in a ratio of numbers of the cells of 10:1, followed by addition of 50% (w/v) polyethylene glycol (PEG e.g. of Boehringer Mannheim, average molecular weight: 4000) to perform cell fusion at 37° C. for 7 minutes. After removal of the supernatant solution (by means of centrifugation), the residue is added with HAT medium (DMEM medium containing 10% fetal bovine serum added with hypoxanthine, aminopterin, and thymidine). The residue has to be suspended so that a concentration of the spleen cells of about 5×10^6 cells/ml is obtained. This cell suspension can than be dispensed and poured into 96-well plastic plates so that one well 15 contains about 100 µl of the suspension, followed by cultivation at 37° C. in 5% carbon dioxide. HAT medium has to be supplemented; for instance in an amount of 50 µl/well on 2nd and 5th days. After that, half volume of the medium can be exchanged every 3 or 4 days in conformity with proliferation of hybridomas.

Screening and Cloning of Hybridomas: Hybridomas, which produce the monoclonal antibody of the present invention, have to be screened for. This has to be done by using, as an index, the inhibitory activity of the monoclonal antibody on the physiological activity possessed by PACAP. Hybridomas, which produced monoclonal antibodies exhibiting reactivity with PACAP's have then to be selected from the selected clones. The obtained hybridomas have then to be transferred to a suitable medium for instance HT medium which is the same as HAT medium except that aminopterin is removed from HAT medium, and cultured further. Cloning can be performed twice in accordance with the limiting dilution method by which stable hybridomas are obtainable.

Production and Purification of Monoclonal Antibodies: 2,6,10,14-Tetramethylpentadecane (e.g. Pristane of Sigma, 0.5 ml) can be intraperitoneally injected into Balb/c female mice (6 to 8 weeks old from the birth). After 10 to 20 days, cells of clones can be (1×10^6 to 10^7 cells) suspended in PBS and intraperitoneally inoculated into

the mice. After 7 to 10 days, the mice can be sacrificed and subjected to an abdominal operation, from which produced ascitic fluid can be collected. The ascitic fluid can be centrifuged to remove insoluble matters, and a supernatant was recovered and stored at ~20° C until purification. Consequently, IgG can be purified from the ascitic fluid supernatant described above by using Hi-Trap Protein-A antibody purification kit (available from Pharmacia, Roosendaal, Netherlands). Namely, the ascitic fluid (2 ml) can be added with Solution A (1.5 M glycine, 3 M NaCl, pH 8.9, 8 ml), and filtrated with a filter for filtration having a pore size of 45 μ m (Millipore). After that, an obtained filtrate can be applied to a column (column volume: 1 ml) charged with Protein Sepharose HP (produced by Pharmacia) sufficiently equilibrated with Solution A, and the column has been washed with Solution A in an amount of 10-fold column volume. Subsequently, an IgG fraction can be eluted with Solution B (0.1 M glycine, pH 2.8) in an amount of 10-fold column volume. The eluted IgG fraction can be dialyzed against PBS. The monoclonal antibodies can be determined for their IgG subclasses by using the purified antibodies obtained in the foregoing, by means of a commercially available subclass-determining kit (trade name: Mono Ab-ID EIA Kit A, produced by Zymed). This method is based on the ELISA method. The Inhibitory Activities of Monoclonal Antibodies can be tested for their possible stimulatory effect on collagen-induced (0.35 μ g/ml) platelet aggregation of human or mouse platelets. A similar approach may be used for the preparation monoclonal antibodies specific to VIP or to PACAP receptor VPAC1 or fragments thereof. Such antibodies can be applied for dosing activating or inhibitory PACAP mimetics by ELISA, for the purpose of monitoring PACAP (analogues) concentrations in the plasma of treated subjects. The VPAC1 have been cloned (Hannar et al, Pharmacol Rev. 50: 265-270, 1998). The human VPAC1 receptor DNA has been characterised from a HT29 human colonic adenocarcinoma cell line library. Human VPAC1 receptor comprises 457 amino acids (Sreedharan et al. Proc. Natl. Acad. Sci. USA 92: 2939- 2943, 1993). The human VPAC1 receptor gene is located on region p22 of chromosome 3 (Sreedharan et al. Biochem Biophys Res Commun 193: 546 – 553, 1995). Cell lines, such *Saccharomyces cerevisiae*, which are naturally devoid of VPAC1, can be transfected to produce such (Hansen MK. Receptors Channels 6: 271 – 281). Vectors for expression of PACAP receptors have been described in WO0107478. The

method for preparation of PACAP receptor proteins has been disclosed in patent application US20020155533.

Preparation of F(ab')2 or monovalent Fab fragments: In order to prepare F(ab')2 fragments, the monoclonal antibody can be dialyzed overnight against a 0.1 mol/L citrate buffer (pH 3.5). The antibody (200 parts) are then digested by incubation with pepsin (1 part) available from Sigma (Saint-Louis, Missouri) for 1 hour at 37°C. Digestion is consequently stopped by adding 1 volume of a 1 M Tris HCl buffer (pH 9) to 10 volumes of antibody. Monovalent Fab fragments can prepared by papain digestion as follows: a 1 volume of a 1M phosphate buffer (pH 7.3) is added to 10 volumes of the monoclonal antibody, then 1 volume papain (Sigma) is added to 25 volumes of the phosphate buffer containing monoclonal antibody, 10 mmol/L L-Cysteine HCl (Sigma) and 15 mmol/L ethylene diaminetetra-acetic acid (hereinafter referred to as EDTA). After incubation for 3 hours at 37°C, digestion is stopped by adding a final concentration of 30 mmol/L freshly prepared iodoacetamide solution (Sigma), keeping the mixture in the dark at room temperature for 30 minutes. Both F(ab')2 and Fab fragments can further be purified from contaminating intact IgG and Fc fragments using protein-A-Sepharose. The purified fragments can finally dialyzed against phosphate-buffered saline (herein after referred as PBS). Purity of the fragments can be determined by sodiumdodecylsulphate polyacrylamide gel electrophoresis and the protein concentration can be measured using the bicinchoninic acid Protein Assay Reagent A (Pierce, Rockford, Illinois).

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~~Haemostasis and thrombosis~~

Claims

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1. Use of an agonist of platelet PACAP receptor for the manufacture of a medicament for the treatment or prevention of a disorder or disease caused by platelet hyperfunction.

10 2. The use of claim 1, wherein the receptor agonist is a VPAC(1) receptor agonist.

3. The use of claim 1, wherein the agonist is PACAP, its analogues or mimetics.

15 4. The use of claim 1, wherein the agonist is PACAP receptor agonists selected from the list consisting of an PACAP, PACAP (1 – 38), [(11,22,28)Ala]-VIP, VIP, [K(15), R(16), L(27)]VIP(1-7)/GRF(8-27), [R(16)]ChSn, [R(16)]-PACAP(1-23), [L(22)]-VIP and (Lys15, Arg16, Leu27)VIP1-7 GRF8-27.

20 5. The use of claim 1, wherein the agonists are PACAP/VIP receptors agonists selected from the group consisting of PACAP 1-38, PACAP1-27, PACAP 2-38, PACAP 2-27, AchIS1 PACAP1-27 and Ala2 PACAP127.

25 6. The use of claim 1, wherein the agonists are amino acid substituted lipophilic VIP derived VPAC receptors agonist.

7. The use of claim 1, wherein the agonists are VPAC(1) agonists selected from the group of compounds consisting of [R(16)]-PACAP(1-23), [(L22)]-VIP, (Lys15, Arg16, Leu27)VIP1-7 GRF8-27.

30 8. The use of claim 1, wherein the disorder or disease caused by platelet hyperfunction is one of thrombus formation.

9. The use of claim 1, wherein the disorder caused by platelet hyperfunction is one of the group consisting of acute coronary syndrome, myocardial infarction, acute myocardial infarction, unstable angina, refractory angina, peripheral vascular disease, 5 occlusive coronary thrombus occurring post-thrombolytic therapy or post-coronary angioplasty, a thrombotically mediated cerebrovascular, syndrome, embolic stroke, thrombotic stroke, transient ischemic attacks, venous thrombosis, deep venous thrombosis, pulmonary embolus, coagulopathy, dissemination intravascular coagulation, thrombosis following angioplasty, restenosis following angioplasty, 10 thrombosis following carotid endarterectomy, thrombotic thrombocytopenic purpura, thromboangiitis obliterans, thrombotic disease associated with heparin-induced thrombocytopenia, thrombotic complications associated with extracorporeal circulation, thrombotic complications associated with instrumentation such as cardiac or other intravascular catheterization, intra-aortic balloon pump, coronary stent, 15 atherosclerosis, or cardiac valve, disorder is characterized by transient ischemic attacks, and conditions required the fitting of prosthetic devices.

10. A pharmaceutical composition, which comprises an amount of PACAP or a mimetic thereof effective for blocking or preventing thrombosis in a subject, and a 20 pharmaceutically effective carrier.

11. The use pharmaceutical composition of claim 10 to manufacture a medicament to treat a subject having a thrombus or at risk of thrombus formation.

Haemostasis and thrombosis

5 ABSTRACT

The present invention relates to a new method for prevention and treatment of either thrombosis or bleeding based on administration of pituitary adenylyl cyclase activating peptide (PACAP) mimetics or inhibitors respectively or by activating or blocking 10 respectively of a platelet VPAC1 receptor.

PCT/EP^{PCT Application}2004/001209



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